Supplemental Document

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Digital labeling for 3D histology: segmenting blood vessels without a vascular contrast agent using deep learning: supplement

MARYSE LAPIERRE-LANDRY,[1] YEHE LIU,[1] MAHDI BAYAT,[2] DAVID L. WILSON,[1,3] AND MICHAEL W. JENKINS[1,4,*]

[1]*Department of Biomedical Engineering, Case Western Reserve University, USA*
[2]*Department of Electrical, Computer and Systems Engineering, Case Western Reserve University, USA*
[3]*Department of Radiology, Case Western Reserve University, USA*
[4]*Department of Pediatrics, School of Medicine, Case Western Reserve University, USA*
[*]*michael.jenkins@case.edu*

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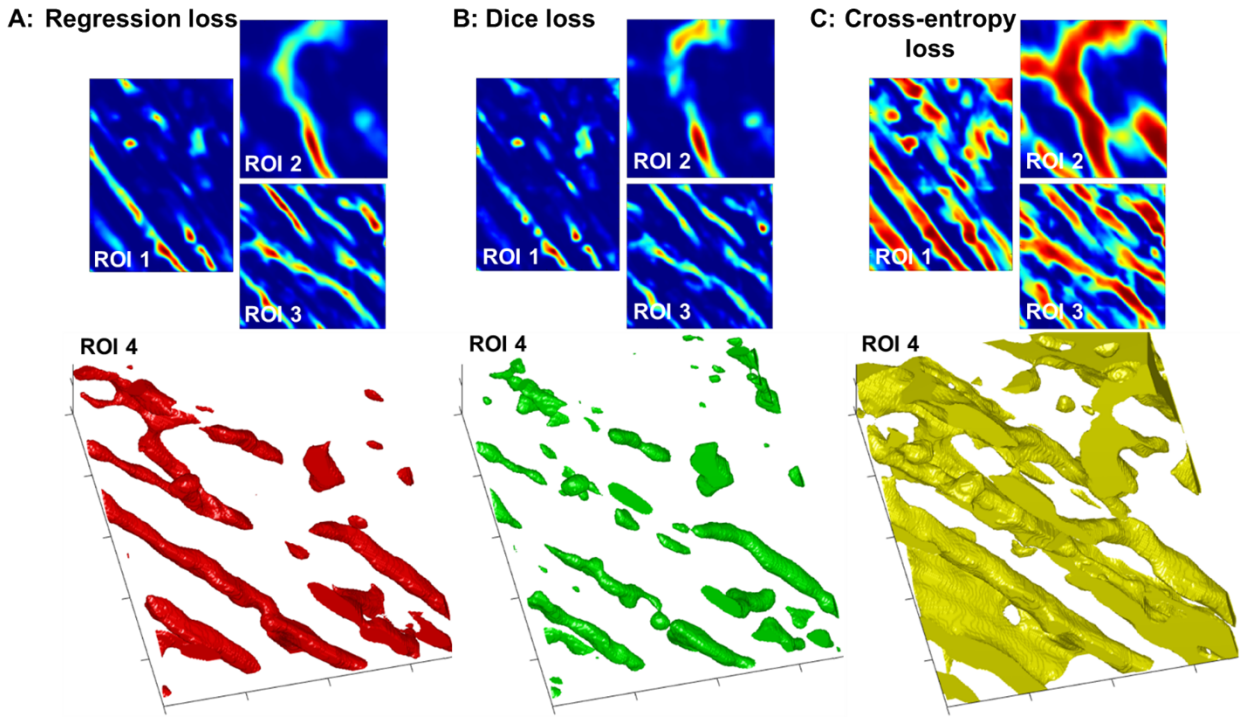
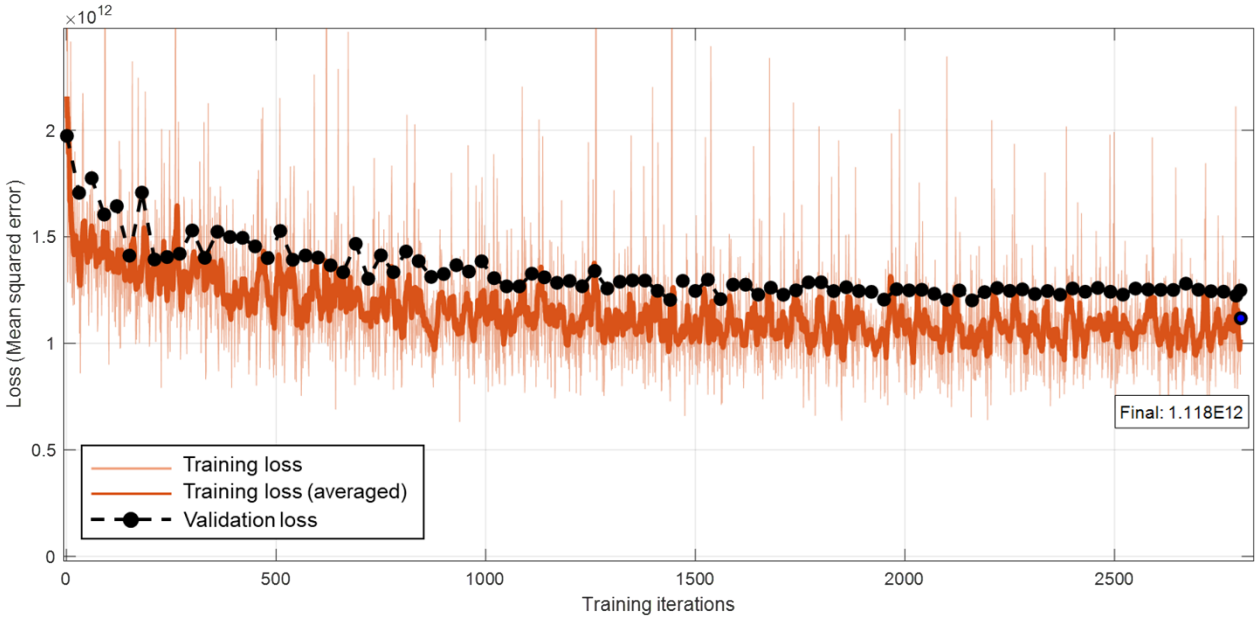
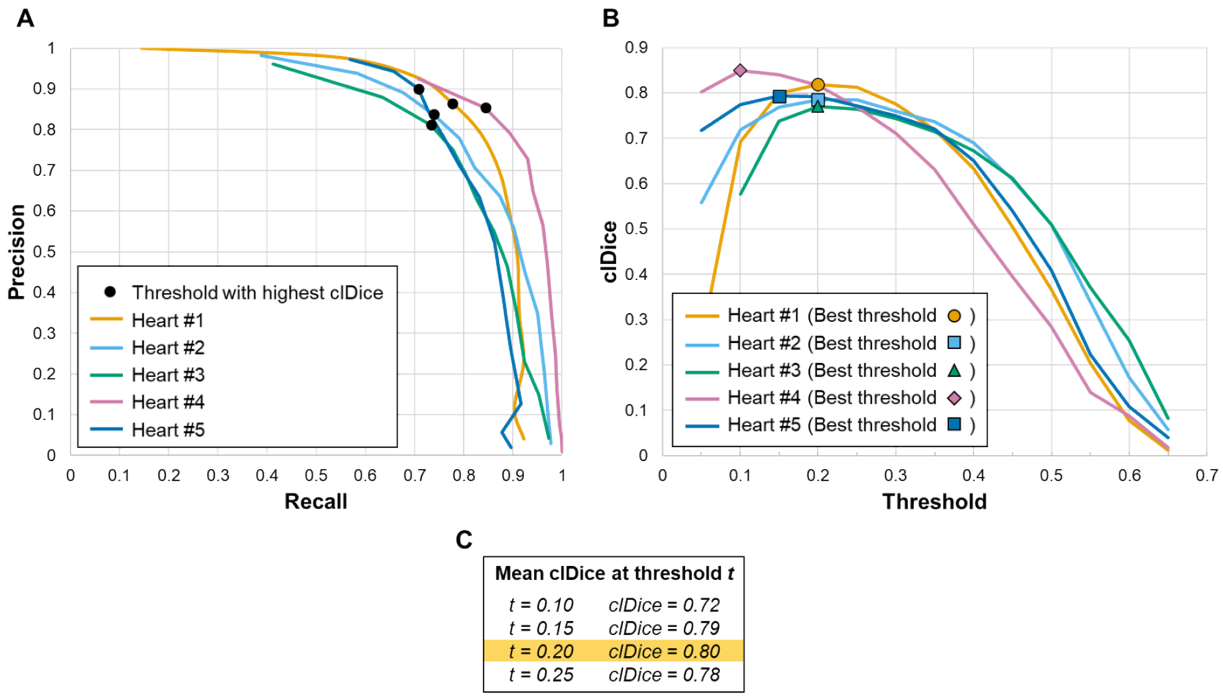


Supplemental Fig. 1: Choice of loss function. Comparison of the raw network prediction for networks trained with **(A)** a regression mean squared error loss, **(B)** a Dice loss, and **(C)** a cross-entropy loss with weight correction favoring the vessel class. For each network, the network prediction with no post-processing is shown for four matching regions of interest (ROI). ROIs #1-3 show mean intensity projections of the raw (un-thresholded) results. ROI #4 shows a three-dimensional rendering of the thresholded prediction over a region. Best thresholds were chosen for each case to minimize false positives and false negatives. More continuous vessels are seen when the network is trained with a regression loss, while disconnected vessels are seen throughout the Dice loss network. The cross-entropy loss network over-segments vessels which leads to large amounts of false positives voxels.



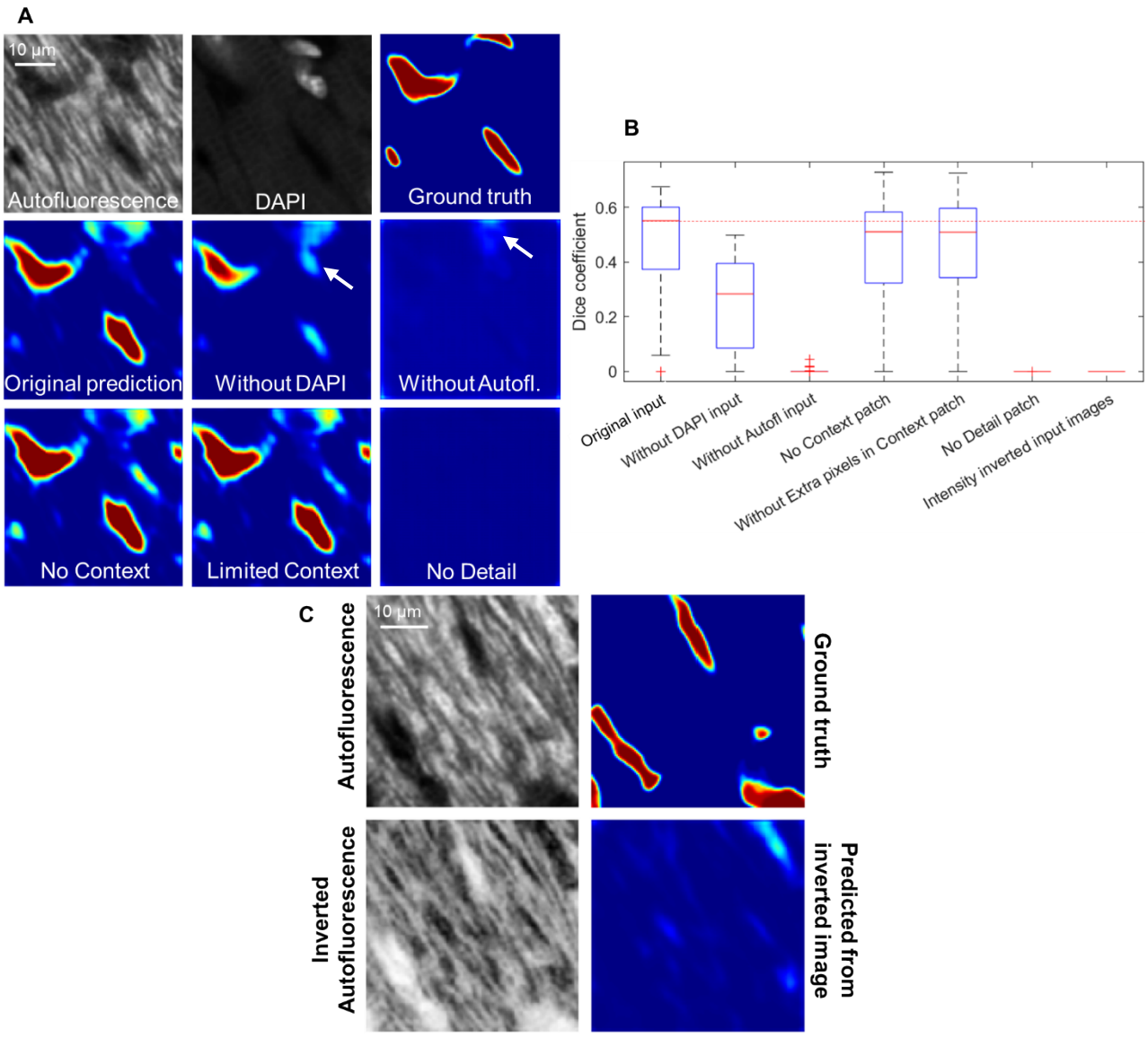
Supplemental Fig. 2: Training and validation loss curves. Loss curves for an example small vessel network, trained for 35 epochs with a mean squared error loss function. The final validation loss is 1.11 x 10^{12}.



C

Mean clDice at threshold *t*	
t = 0.10	*clDice* = 0.72
t = 0.15	*clDice* = 0.79
t = 0.20	*clDice* = 0.80
t = 0.25	*clDice* = 0.78

Supplemental Fig. 3: Determination of the segmentation threshold for vessels. (A) Precision-Recall curves for each heart, where each data point indicates the recall (as defined by Eq. 2) and the precision (as defined by Eq. 3) obtained for a certain threshold value to separate background voxels from vessels voxels. The threshold with the highest clDice score is indicated for each heart. **(B)** clDice scores as a function of threshold values for all five hearts, with the threshold leading to the highest clDice score indicated for each heart. **(C)** Mean clDice scores calculated for all five hearts at different threshold values *t*. The highest clDice score is obtained with a threshold value of 0.2.



Supplemental Fig. 4: Role of different inputs onto the HEUnet predictions. (A) Example input patches (autofluorescence and DAPI), with corresponding ground truth, network prediction ("original prediction"), and network outputs following modification to the input patches. When the DAPI input is replaced by zeros, the network predicts vessels where nuclei are present (white arrow). When the autofluorescence input is replaced by zeros, the network does not predict any vessels. There is some activation where the nuclei are present (white arrow). When the "context" patch is replaced by all zeros, the network predicts vessel position but is more likely to make mistakes. This is similar to a limited case where all extra pixels of the "context" patch are replaced by zeros but the center of the patch is maintained. No predictions are made when the "context" patch is provided to the network while the "detail" patch is replaced by zeros. **(B)** Dice coefficients obtained over 30 random image patches for the different conditions. **(C)** Network prediction made on an image with inverted pixel intensity. The network activates for low-intensity pixels, but with low certainty, as the shape of the region does not match the shape of vessels.